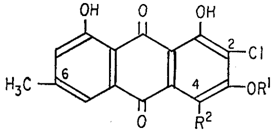
Itiro YOSIOKA*, Hiroshi YAMAUCHI*, Kimiko MORIMOTO*
and Isao KITAGAWA*:

The pigment constituents of some *Anaptychia* species

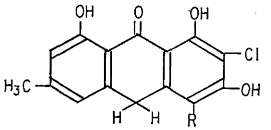
吉岡一郎*・山内 博*・森元君子*・北川 勲*:

ゲジゲジゴケ属地衣数種の色素成分

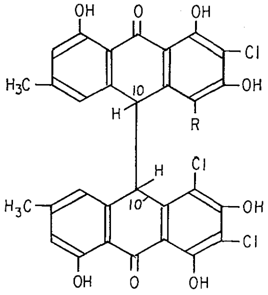
As a continuation of the chemical study on the lichen substances, we have been engaged in the structural investigation of the coloring matters originated from a lichen, *Anaptychia obscurata* (Nyl.) Vain.** In the previous reports1,2), we described the isolation of emodin (0.002%)***, and six new coloring substances, designated tentatively as AO-1 (0.06%), AO-2 (0.009%), AO-3



I: $R^1=R^2=H$: AO-1
II: $R^1=H$, $R^2=Cl$: AO-2
IV: $R^1=CH_3$, $R^2=H$: fragilin



III: R=H: AO-1-anthrone
VIII: R=Cl: AO-2-anthrone



V: R=H: flavoobscurin A (AO-4)
VI: R=Cl: flavoobscurin B_1 (AO-5)
VII: R=Cl: flavoobscurin B_2 (AO-6)
(VI, VII: isomeric at C_{10} and/or $C_{10'}$)

Fig. 1. Chemical structures of pigments isolated from *Anaptychia* species.

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* Faculty of Pharmaceutical Sciences, Osaka University, Toyonaka, Osaka. 豐中市刀根山 大阪大学薬学部

** Collected at Kirii, Gifu Prefecture.

*** The respective yields based on the air-dried lichen are given in the parentheses.

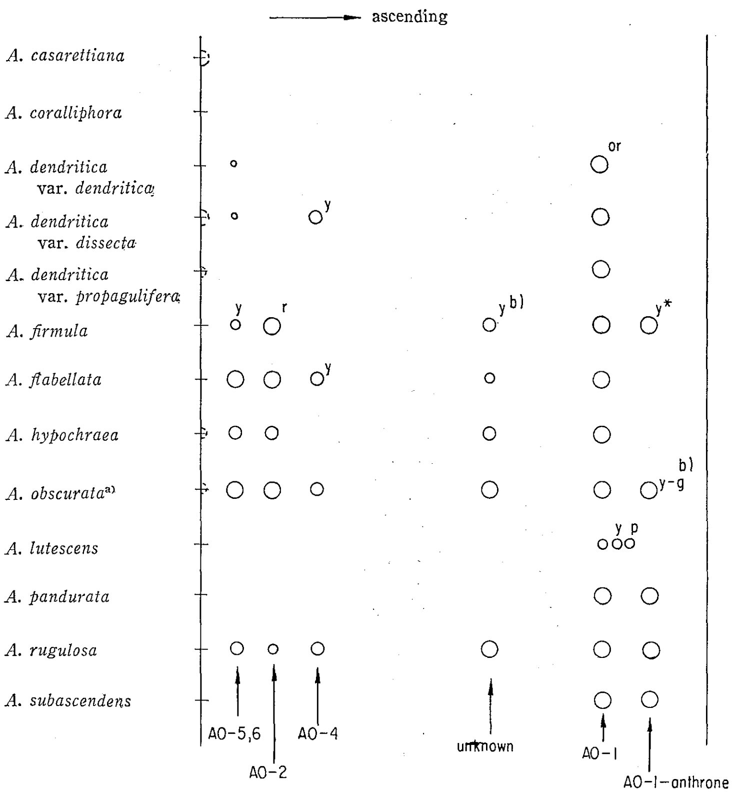

Fig. 2. Thin layer chromatogram on silica gel (Camag) by using chloroform+methanol (4:1) as a developing mixture. y: yellow, or: orange, r: red, y-g: yellowish green, p: pink, y*: yellow spot on running TLC, but turns to pink quickly upon standing. The coloration by $Mg(OAc)_2$ is uncertain. a) The spot due to AO-3 is not detected on TLC of the total extract. b) The coloration of spot due to AO-1-anthrone and unknown substance (presumably AO-2-anthrone) varies to greenish brown and green upon standing respectively.

(0.0065%), AO-4, -5, -6 (totally ca. 0.1%), by virtue of the ordinary alkaline fractionation of the ether extract of the lichen followed by the successive column chromatography on silicic acid and elucidated the chemical structures except AO-3 as shown below. These newly isolated six pigments except AO-3 are characteristic due to the chlorine-containing structures.

AO-1 (I), (orange needles), mp. 286-287°, and AO-2 (II), (orange needles), mp. 267-269°, correspond to monochloro- and dichloro-emodins. It is interesting to note that AO–1 has also been isolated from a fungus, *Aspergillus fumigatus* (J–4) by Yamamoto *et al.*[3] independently in addition to AO–1-anthrone (III). As for the related investigation, a fungus *Penicillium nalgiovensis* Laxa has been known to produce a monochloro-anthraquinone derivative named nalgiolaxin, whose constitution has recently been established[4]. In the lichen family, Bruun *et al.*[5] elucidated the structure of fragilin as 2-chloro-parietin (IV), isolable from *Sphaerophorus fragilis* (L) Ach. (=*S. fragilis* Pers.) and *S. globosus* Vain (=*S. coralloides* Pers.), while the isola-

Table 1. Results of thin layer chromatographic tests.

– : not detected. ± : detected, but in weak spot. + : detected in medium spot. ⧺, ⧻ : detected in significant spot. a) Presumably assumed to be AO–2-anthrone. b) Unseparable on TLC under the condition described here, but separable by using chloroform +methanol mixture (2 : 1) as a developing solvent system. c) Pigments detected on TLC other than the compounds mentioned in this paper. d) Two spots, yellow and pink, and the latter showed orange coloration by $Mg(OAc)_2$.

	monomeric				dimeric		others[c]
	AO–1	AO–1-anthrone	AO–2	unknown[a]	AO–4	AO–5 & 6[b]	
A. casarettiana	–	–	–	–	–	–	trace
A. coralliphora	–	–	–	–	–	–	–
A. dendritica var. *dendritica*	+	–	–	–	–	±	–
A. dendritica var. *dissecta*	⧺	–	–	–	+	±	±
A. dendritica var. *propagulifera*	+	–	–	–	–	–	–
A. firmula	⧺	–	+	+	–	+	⧺
A. flabellata	⧺	–	+	+	+	⧺	–
A. hypochraea	⧺	–	+	+	–	+	–
A. obscurata	⧻	+	+	⧺	+	⧺	–
A. lutescens	±	–	–	–	–	–	± ±[d]
A. pandurata	⧺	⧺	–	–	–	–	–
A. rugulosa	⧺	⧺	+	⧺	+	+	–
A. subascendens	⧺	⧺	–	–	–	–	–

tion of AO-1 and fragilin from *Nephroma laevigatum* Ach. non. auct. nonn. (=*N. lusitanicum* Schaer.) was reported by Bendz *et al.*6) recently. These are the only examples of chloro-anthraquinone derivatives appeared in the literature of natural products heretofore. AO-2 consequently, is the first appearance of possessing two chlorine atoms in its molecule among the naturally occurring anthraquinone derivatives.

AO-3 (orange needles), mp. 269-271° (decomp.) is a quinonic compound having hydroxyl but lacking halogen, whose structure not yet been clarified.

Three other pigments, AO-4 (V) (greenish yellow needles), mp.>360° (darkening from 195°), $[\alpha]_D+30°$, AO-5 (VI) (yellow crystals), mp.>360° (darkening from 210°), $[\alpha]_D+35°$, and AO-6 (VII) (yellow needles), mp.>360° (darkening from 215°), $[\alpha]_D+7°$, now named flavoobscurin A_1, B_1, and B_2 respectively, belong to the bisanthronyl (10,10′) group possessing three or four chlorine atoms in their compositions and the latter two (B_1 and B_2) are found isomeric each other. These pigments exhibit yellow spots on the thin layer chromatogram (TLC), which are fairly sensitive to light and change gradually to greenish upon standing for a while. It is noteworthy to point out here that they are the first occasions as chlorine-containing bisanthronyl derivatives in nature.

On the other hand, the survey of the certain coloring substances has been known as the important characteristic of the taxonomical criterion in *Anaptychia* species7). Therefore, the above mentioned establishment of several unique chlorine containing anthraquinone analogues in *Anaptychia obscurata* has enabled us to survey the pigment compositions of some other related *Anaptychia* species in this connection.

Thus, thirteen species*, whose pigment compositions have yet been unclarified, such as: *A. casarettiana* Mass., *A. coralliphora* (Tayl.) Vain., *A. dendritica* (Pers.) Vain. var. *dendritica*, *A. dendritica* (Pers.) Vain. var. *dissecta* (Kurok.) Kurok., *A. dendritica* (Pers.) Vain. var. *propagulifera* Vain., *A. firmula* (Nyl.) Dodge et Awas., *A. flabellata* (Fée) Mass., and *A. hypochraea* Vain., *A. lutescens* Kurok., *A. obscurata* (Nyl.) Vain., *A. pandurata* Kurok., *A. rugulosa* Kurok., *A. snbascendens* Asah., have been surveyed by TLC using the ether immersion of the lichens and the above described pigments as the standards. The results are summarized in Fig. 2 and Table 1.

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* Preserved at the Herbarium of the National Science Museum, Tokyo.

The coloring constituents of *A. casarettiana* and *A. coralliphora* were not detected by the present procedure, suggesting them to be other type of pigments than those mentioned here, while *A. lutescens* was revealed to contain only trace amount of AO-1 and some other coloring substances.

The rest of ten species was found to contain the considerable amount of AO-1 and the related pigments belonging to hydroxy-anthraquinone analogues as described above. It was also disclosed that *A. firmula* contains an additional orange pigment appearing at the higher Rf value than AO-1 on TLC.

Finally it should be mentioned that AO-1-anthrone (III), whose isolation unsuccessful in our earlier work, has been found to exist not only in *A. obscurata*, but in *A. pandurata*, *A. rugulosa*, and *A. subascendens* in the fair amount, and furthermore, the yellow green spot appearing between AO-1 and AO-4, although unidentified with the authentic sample (for not available yet), seems presumably ascribable to AO-2-anthrone (VIII), whose confirmation of course awaits the further examination.

The authors wish to express their sincere thanks to Dr. S. Kurokawa, the National Science Museum, Tokyo, for his generous admission to work on the precious herbarium of the Museum and also for his fruitful discussion throughout the study. They also thank cordially Prof. Y. Yamamoto, Kanazawa University, for his kind gift of AO-1-anthrone.

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地衣 *Anaptychia obscurata* (Nyl.) Vain. の色素成分の化学的研究によりその構造が明らかになった 5 種の新しい含塩素アンスラキノン AO-1 (I), AO-2 (II) 及び含

塩素ビスアンスロニル誘導体 AO-4 (V), AO-5 (VI), AO-6 (VII) を標品として，更に13種（うち1種は上記と同種で採集地が異る）の *Anaptychia* 属地衣のエーテル抽出物について薄層クロマトグラフィーによる検索を行った。この結果，*A. casarettiana, A. coralliphora* の色素含量は少く薄層上顕著なスポットを与えない。*A. lutescens* は AO-1 を微量含有する以外に2種の別種色素を少量含有することが判明した。残りの10種類即ち，*A. dendritica* var. *dendritica, A. dendritica* var. *dissecta, A. dendritica* var. *propagulifera, A. firmula, A. flabellata, A. hypochraea, A. obscurata, A. pandurata, A. rugulosa, A. subascendens* ではいずれも AO-1 を相当量含有することがわかった。又，上記の他の色素の含有量は Fig. 1 と Table 1 に示すとおりである。更に，AO-1-anthrone (III) 及び AO-2-anthrone (VIII) と思われるスポットを数種の地衣について確認しその含有量もかなりの量であることが判明した。

○地衣類思い出話 (3)（富樫　誠）Makoto TOGASHI: Miscellaneous notes on lichens or lichenological survey (3)

最近 *Usnea* 研究の面で問題となった件は *Usnea creberrima* Vain. の再発見である。事の起りは此の名の地衣が *Euusnea* 亜属に入れてあるにも不拘，そのタイプ標本を検査された朝比奈先生は此れが中空の中軸を持つ事で *Eumitria* 亜属に入るものでなければならず，又先生の *Eumitria* 類の標本にはこれと同定する様なものがないので，故安田篤氏が採集した仙台の東部太平洋海岸を捜せと云う事になり，昭和40年の3月と5月の2回，陸前・閖上浜一帯南北約10キロに亘る地帯を東北大薬学科升本教授の応援もあって大々的に採集し，*Eumitria* と考えられる地衣は根子スギ採集したが，多少タイプに似たものはあっても，タイプそっくりと云う標本はなかった。然し幸にしてヒマラヤ採集品中にタイプそっくりの標本が若干あったので一応ケリがついた（本誌 41: 161, 1966)。然し，残た問題は先生が先に一応 *U. creberrima* に充てた標本を何と呼ぶかと云う件で先生を煩わしたが，これも大部分 *U. roseola* Vain. の一型に充当して方がついた。最初の命名者 Vainio の間違は兎も角，あの鋭い観察眼のある Motyka 迄 *Eumitria* 所属を見逃して居たことは不思議のことと云えよう。